surfaces we evaluate the effect of a circular permutation on the free energy landscape for the protein T4 lysozyme. We observe changes which, while subtle, largely affect the communication, or cooperativity, between the two domains which has been experimentally observed. The free energy landscapes show that both the wild type and circular permutant have an on-pathway intermediate, whose existence is confirmed by experiments, where one of the domains is completely formed. The landscapes, however, differ in the position of the rate-limiting step for folding, which occurs before the intermediate in the wild-type and after in the circular permutant. This shift of transition state explains the observed change in the cooperativity. The underlying free energy landscape thus provides a microscopic description of the folding dynamics and the connection between circular permutation and the loss of cooperativity experimentally observed.

291-Pos Board B77

A Hydrodynamic Fast Mixer Utilizing 3D Focusing to Follow Protein Folding Kinetics

Kelly S. Burke, Dzmitry Parul, Brian Dyer.
Emory University, Atlanta, GA, USA.

We have developed a hydrodynamic fast mixer capable of following protein folding kinetics. Our design, which comprises two co-centered fused silica capillaries, employs three dimensional focusing of the sample channel to create a laminar flow. Construction of the mixer is simple and inexpensive, requiring no specialized equipment or techniques. In characterizing this new mixer, we have also developed a novel sample flow rate calibration method utilizing the fluorescence decay of excited europium (Eu) beads. To demonstrate the mixer's application in biophysical studies, we have used this mixer to study apomyoglobin (apoMb) folding kinetics by inducing folding or unfolding via a rapid change in the pH of the protein's environment. We probed the protein with 1-anilino-8-naphthalene sulfonate (1,8-ANS), and observed the fluorescence using fluorescence confocal microscopy. Scans along the z-axis of the sample channel were collected and analyzed to identify the location of folding or unfolding events and timescale over which these events occurred. Our ultimate goal is to develop a kinetic model of apoMb folding. If the model agrees with previous work on apoMb folding kinetics, we may then use our mixer to characterize the folding and reaction kinetics of other protein systems.

292-Pos Board B78

Energetics of Protein Folding Kinetics

Huseyin Kaya[1], Zeynep Uzunoglu[2].
[1]Gaziantep University, Gaziantep, Turkey, [2]Ataturk University, Erzurum, Turkey.

Experimentally observed diversity in protein folding rates for single domain two-state proteins is very challenging phenomena. Although these two-state proteins have comparable chain sizes, the difference in their folding rates between the slowest and fastest ones could be 9 orders of magnitude. in this study, native-centric C_alpha based course-grained molecular dynamics protein models with Langevin dynamics, which contain different protein energetics such as homogenous and heterogenous interactions, short and long range non-local interactions, local interactions, non-native repulsive interactions, have been considered. The folding kinetics of a protein set consists of 26 experimentally studied two-state proteins was examined. We have shown that, besides the topology of the proteins, presence of non-native repulsive interactions and range of non-local interactions are crucial to observe experimental-like high diversity in the folding rates. Among the protein models we have considered, the one which includes heterogenous and short range non-local interactions, and non-native repulsive interactions, provided a significant correlation between experimental and computational folding rates, i.e., $r = 0.68$. in addition, for the same model and protein set, an excellent agreement in the diversity of folding rates comparing with experimental results, e.g 5 orders of magnitude, was observed. Contributions of each energetics on protein dynamics will also be discussed in details.

293-Pos Board B79

Complete and Reversible Chemical Denaturation of an α-Helical Membrane Protein

Sebastian Fiedler, Jana Broecker, **Sandro Keller**.
University of Kaiserslautern, Kaiserslautern, Germany.

The question of how an unordered polypeptide chain assumes its native, biologically active conformation is one of the greatest challenges in molecular biophysics and cell biology. This is particularly true for membrane proteins. Chemical denaturants such as urea have been used successfully for in vitro un- and refolding studies of soluble proteins and β-barrel membrane proteins. in stark contrast with these two protein classes, in vitro unfolding of α-helical membrane proteins by urea is often irreversible, and alternative denaturation assays using the harsh detergent sodium dodecyl sulphate suffer from a lack of a common reference state.

Here we present the complete and reversible chemical denaturation of the bacterial α-helical membrane protein Mistic out of different micellar environments by urea. We applied multidimensional spectroscopy and techniques typically used in β-barrel membrane protein unfolding. Mistic unfolds reversibly following a two-state equilibrium that exhibits the same unfolded reference state. This allows for a direct comparison of the folding energetics in different membrane-mimetic systems and contributes to our understanding of how α-helical membrane proteins fold as compared with both β-barrel membrane proteins and water-soluble proteins.

294-Pos Board B80

Disulfide-Driven Assembly and Aggregation of the P2X Receptor

Devid El-Wahsch, Ralf Hausmann, Michaela Schumacher, Ursula Braam, Silvia Detro-Dassen, **Gunther Schmalzing**.
RWTH Aachen University, Aachen, Germany.

P2X receptors are cation channels gated by extracellular ATP that are formed by the non-covalent assembly of three identical or homologous subunits. All P2X subunits contain ten strictly conserved cysteine residues (denoted here with sequential numbers, C1-C10) in the ectodomain that give rise to five intrachain disulfides per subunit. To examine the role of the cysteine residues in the folding and assembly of the P2X1 receptor, we took advantage of the reducing agent dithiothreitol (DTT) that inhibits formation of disulfides in living cells without perturbing most other functions (Braakman et al. EMBO J. **11**, 1717-1722, 1992). in intact *X. laevis* oocytes, DTT kept newly synthesized P2X1 subunits in their less compact reduced form that aggregated, but did not trimerize or reach the plasma membrane. Upon DTT washout, the preformed P2X1 subunits oxidized into the more compact form and homotrimerized efficiently to form a fully functional ATP-gated receptor that appeared at the plasma membrane. in the mature homotrimeric state, the P2X1 receptor was DTT resistant. The impact of individual cysteine residues on homotrimerization was assessed by studying cysteine-to-serine single mutants. The resulting presence of an odd number of nine cysteine residues in the ectodomain significantly increased aggregate formation. Depending upon which residue was mutated, homotrimerization was impaired (C1S to C6S) or abolished (C7S to C10S), resulting in reduced or absent cell surface expression, respectively. in contrast to the wt rP2X1 receptor, the ectodomain of mutants C1S to C6S contained a maleimide-reactive thiol, indicating invariant accessibility of the unpaired cysteine residue. These results will be discussed in relation to the crystal structure of the zebrafish P2X4 receptor (Kawate et al. Nature **460**, 592–599, 2009).

295-Pos Board B81

Role of the N- and C-Terminal Helices in Apolipophorin III Stability and Lipid Binding

Pankaj Dwivedi, Johana Rodriguez, Paul M.M. Weers.
California State University, Long Beach, LONG BEACH, CA, USA.

Apolipophorin III (apoLp-III) from the insect *Locusta migratoria* is a model apolipoprotein to study their structure-function relationship. In contrast to the common 4-helix bundle motif, apoLp-III is a bundle of five amphipathic α-helices. We hypothesized that the 5-helix bundle of apoLp-III may have evolved to modulate lipid-binding activity. To verify this, N- and C-terminal helix deletion mutants were designed. The N-terminal deletion mutant was expressed and purified from *E.coli*. However, bacteria did not produce the C-terminal helix deletion mutant. Therefore, valine 131, which resides in the loop connecting helix-4 and −5, was replaced by methionine. Cleavage by CNBr then produced the C-terminal deletion variant. in addition, five truncation mutants in the C-terminal α-helix were engineered by introducing stop codons at Q136, E140, E144, Q150 and Q154, to create shortened versions of the protein. The mutant proteins truncated at E144, Q150 and Q154 were successfully expressed and purified but no protein was produced when apoLp-III was truncated at Q136 or E140. Guanidine denaturation analysis showed a decreased stability for the N- and C-terminal helix deletion mutants as indicated by the midpoint of denaturation (0.19 and 0.23 M for the deletion mutants and 0.50 M for wild-type apoLp-III). Truncation at E144, Q150 and Q154 also resulted in a decrease in protein stability with midpoints of 0.23, 0.35, and 0.36 M, respectively. Far UV circular dichroism showed a significant reduction in α-helical content for all mutants. Lipid binding analysis using dimyristoylphosphatidylcholine vesicles showed that the mutant proteins formed discoidal protein-lipid complexes at much higher rates compared to wild-type apoLp-III. The results show that deletion of a terminal α-helix result in an increased lipid binding but this comes at the cost of a decrease in protein stability.